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Interferon in lyssavirus infection

Die Rolle des Interferon in der Lyssavirus-Infektion

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Summary

Rabies is a zoonosis still claiming more than 50 000 human deaths per year. Typically, human cases are due to infection with rabies virus, the prototype of the *Lyssavirus* genus, but sporadic cases of rabies-like encephalitis caused by other lyssaviruses have been reported. In contrast to rabies virus, which has an extremely broad host range including many terrestrial warm-blooded animals, rabies-related viruses are associated predominantly with bats and rarely infect terrestrial species. In spite of a very close genetic relationship of rabies and rabies-related viruses, the factors determining the limited host range of rabies-related viruses are not clear. In the past years the importance of viral countermeasures against the host type I interferon system for establishment of an infection became evident. The rabies virus phosphoprotein (P) has emerged as a critical factor required for paralysing the signalling cascades leading to transcriptional activation of interferon genes as well as interferon signalling pathways, thereby limiting expression of antiviral and immune stimulatory genes. Comparative studies would be of interest in order to determine whether differential abilities of the lyssavirus P proteins contribute to the restricted host range of lyssaviruses.

Keywords: zoonosis, innate immunity, inflammation, antiviral

Zusammenfassung

Die Tollwut ist eine Zoonose, der auch heute noch jährlich mehr als 50 000 Menschen zum Opfer fallen. Menschliche Krankheitsfälle werden in der Regel durch eine Infektion mit dem Rabies Virus (Tollwutvirus), dem Prototyp des Genus *Lyssavirus*, verursacht, wobei einzelne Fälle von Tollwut-Enzephalitis jedoch auf andere Lyssaviren zurückzuführen sind. Im Gegensatz zum Rabies Virus, welches ein extrem breites Wirtsspektrum besitzt, das unter anderem zahlreiche terrestrische Säugetiere umfasst, sind Rabies-verwandte Viren hauptsächlich mit Fledermäusen assoziiert und infizieren nur selten terrestrische Spezies. Obwohl Rabies und Rabies-verwandte Viren genetisch sehr ähnlich sind, sind die Faktoren, die das Wirtsspektrum der Rabies-verwandten Viren limitieren, nicht bekannt. Wie in den vergangenen Jahren gezeigt wurde, sind virale Mechanismen gegen das Typ I Interferonsystem für die Etablierung einer Infektion notwendig. Das Rabies Virus Phosphoprotein (P) wurde als wesentlicher Faktor identifiziert, der für die Hemmung der transkriptionellen Aktivierung der Interferon Gene und der Interferon-vermittelten Signalübertragung notwendig ist. Dadurch wird die Expression von antiviralen und immunstimulatorischen Genen begrenzt. Die Durchführung von vergleichenden Studien wäre interessant, um aufzuklären, ob unterschiedliche Wirksamkeiten der Lyssavirus P Proteine zur Einschränkung des Wirtsspektrums beitragen.

Schlüsselwörter: Zoonose, angeborene Immunität, Entzündung, antiviral

The *Lyssavirus* genus

Rabies is a feared zoonotic infectious disease long known to mankind (Theodorides, 1986). The main causative agent is rabies virus (RABV), the prototype of the *Lyssavirus* genus in the *Rhabdoviridae* family [rhabdos; greek: rod]. The rhabdoviruses are non-segmented negative-strand RNA viruses (NNSV; order *Mononegavirales*) characterized by a typical bullet- or rod-shaped morphology and comprise viruses infecting a broad host range, including plants, insects, fish, and other vertebrates (Fu, 2005). While mammalian rhabdoviruses include several economically important livestock pathogens such as vesicular stomatitis virus (VSV; *Vesiculovirus* genus) or bovine ephemeral fever virus (BEFV; *Ephemerovirus* genus), a constant and global threat for human health is RABV.

As for other rhabdoviruses, the lyssavirus particles are composed of a stable, helical nucleocapsid or ribonucleoprotein (RNP) comprising the 12 kb negative sense genome RNA and a lipid envelope which contains glycoprotein spikes that mediate entry into host cells. The viral RNA comprises five genes in the conserved order 3′-N-P-M-G-L-5′ encoding the nucleoprotein (N), which tightly encloses the RNA, the phosphoprotein (P), a cofactor for the RNA polymerase and a chaperone for RNA encapsidation by N, the matrix protein (M), which is critical for virus assembly and budding, and for regulation of RNA synthesis, the transmembrane spike glycoprotein (G), and the "large" protein (L), which is the catalytic subunit of the viral RNA polymerase.

RABV is endemic almost worldwide in wildlife, including diverse species such as foxes, raccoons, raccoon dogs, skunks, jackals, mongooses, as well as in dogs. Also bats are suitable reservoirs for RABV, as illustrated in the Americas. While the availability of inactivated vaccines for humans and pets, live attenuated viruses for wildlife, and efficient post-exposure treatments has allowed control of rabies in developed countries, rabies encephalitis still causes more than 50 000 human deaths each year in developing countries where canine rabies is endemic (Warrell and Warrell, 2004).

In addition to RABV, the *Lyssavirus* genus comprises an increasing number of virus species most of which are causative agents of rabies-like encephalitis. In contrast to RABV, non-rabies lyssaviruses are mainly associated with bats, but incidents were reported of transmission to rodents, dogs, cats, and other animals, many of these viruses even show potential to cause human rabies (Johnson et al., 2010; WHO, 2005). This indicates a limited susceptibility of terrestrial hosts to bat viruses, which is the first prerequisite to further adaptation to novel host species.

Along with RABV, ten other virus species have been assigned to the *Lyssavirus* genus so far, and a further two (Shimoni bat virus [SHIBV], Bokeloh bat virus [BBLV]) were proposed to be included (Tab. 1). Phylogenetic analyses suggest that all lyssaviruses, including RABV and Mokola virus (MOKV), which so far has been isolated only from terrestrial animals, originated from bats and that RABV and probably MOKV have genetically adapted to terrestrial animals during evolution.

Lyssaviruses are currently classified in seven genotypes and two phylogroups according to similarities of sequence and antigenicity (Badrane et al., 2001) (Tab. 2). Phylogroup I comprises viruses of the genotypes 1, 4, 5, 6, and 7; and phylogroup II members of genotypes 2 and 3. SHIBV was recently grouped into phylogroup II and due to differences to LBV it was suggested to be classified in a new genotypic subgroup (Kuz'min et al., 2010). Due to greater divergence, West Caucasian bat lysavirus (WCBV) was suggested to be included into a new phylogroup III. However, for WCBV, SHIBV, Aravan virus (ARAV), Khujand virus (KHUV), and BBLV, characterization is still ongoing. Importantly, vaccinations against RABV appear to be effective against other viruses

TABLE 1: *Members of the Lyssavirus genus listed according to their genetic relation to RABV. Host species, country, and year of first isolation are provided. Classification in genotypes and phylogroups as assigned so far*

Virus	First Isolation	Distribution	GenBank accession no.	Geno-type	Phylogroup
RABV	(PV strain), cow France, 1882	Worldwide	M13215.1	1	I
ABLV	Bat *Pteropus alecto*, Australia, 1996	Australia	AF006497	7	I
ARAV	Bat *Myotis blythi*, Kyrghyzstan, 1991	Kyrghyzstan	EF614259		
EBLV-2	Human, Finland, 1986	Europe	EF157977	6	I
KHUV	Bat *Myotis mystacinus*, Tajikistan, 2001	Tajikistan	EF614261		
BBLV	Bat *Myotis nattererii*, Germany, 2009	Germany	AEL79467.1		
IRKV	Bat *Murina leucogaster*, Russia, 2002	Eastern Siberia	EF614260		
DUVV	Human, South Africa, 1970	Southern Africa	EU293119	4	I
EBLV-1	Bat *Eptesicus serotinus*, Germany, 1986	Europe	EF157976	5	I
MOKV	Shrew, Nigeria, 1968	Sub-Saharan Africa	AY062074	3	II
LBV	Bat *Eidolon helvum*, Nigeria, 1956	Sub-Saharan Africa	U22842	2	II
SHIBV	Bat *Hipposideros commersoni*, Kenya, 2009	Kenya	GI291195467		
WCBV	Bat *Miniopterus schreibersi*, Russia, 2002	Western Caucasus Mountains	EF614258		(III)

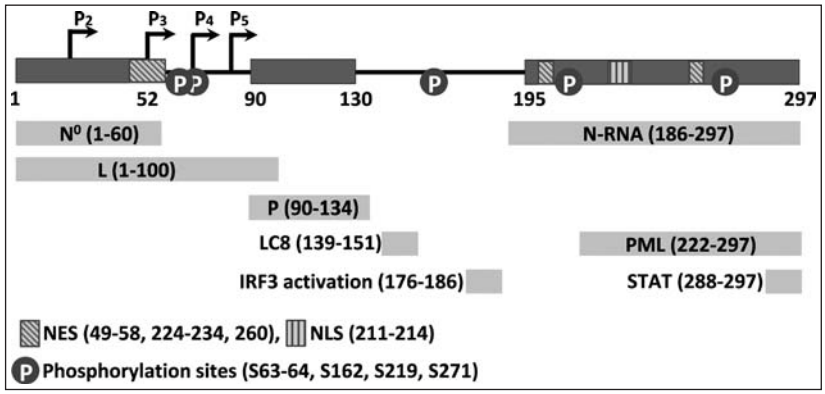

FIGURE 1: *RABV phosphoprotein is a multifunctional protein comprising three structured domains (dark grey), an N-terminal domain (aa 1–52), central domain (aa 90–130), and C-terminal domain (aa 195–297), which are connected via two intrinsically disordered regions (black lines) (Gerard et al., 2009). The start codons of N-terminally truncated P forms P2–5, NLS and NES sites, and phosphorylation sites are indicated. Depicted in light grey bars beneath the protein are distinct domains responsible for binding to the indicated proteins or with functional relevance.*

of phylogroup I, but not against phylogroup II or III viruses (Badrane et al., 2001). This could be explained by differences in the glycoprotein (G), which is the main target of virus neutralizing antibodies.

Phylogenetically, the closest relatives to RABV are the phylogroup I viruses Australian bat lyssavirus (ABLV) (Fraser et al., 1996), Aravan virus (ARAV) (Kuz'min et al., 1992), Khujand virus (KHUV) (Kuz'min et al., 2002), European bat lyssavirus 2 (EBLV-2) (Lumio et al., 1986), and the recently isolated BBLV (Freuling et al., 2011) while the remaining phylogroup I viruses comprising Irkut virus (IRKV) (Botvinkin et al., 2003), Duvenhage virus (DUVV) (Meredith et al., 1971) and EBLV-1 (Schneider et al., 1985) are less closely related. Distantly related are the phylogroup II viruses Lagos bat virus (LBV) (Boulger and Porterfield, 1958), MOKV (Shope et al., 1970), and SHIBV (Kuz'min et al., 2010). The lyssavirus most divergent from RABV is West Caucasian bat virus (WCBV) (Botvinkin et al., 2003). As suggested by the cases of RABV and MOKV, the potential of bat-borne lyssaviruses to adapt to terrestrial hosts seems to reside in both phylogroups (Johnson et al., 2010).

The genetic factors important for adaptation of lyssaviruses to other species have not been determined so far, although several candidates are close at hand. They include the single surface glycoprotein (G) gene, which in RABV is a major factor determining neurotropism and neuroinvasiveness. Specifically, the G protein is responsible for attachment to receptors on neuronal cells (Lafon, 2005), retrograde transport of viral particles (Klingen et al., 2007), transsynaptic spread (Wickersham et al., 2007b; Etessami et al., 2000) and preservation of neuronal integrity (Prehaud et al., 2010), which all is required for gaining access to the CNS, where the viruses multiply extensively (Dietzschold et al., 2008). In addition, species-specific factors associating with the viral RNA synthesis machinery (which comprises the viral N, P, M, and L proteins) may limit the efficiency of replication in a heterologous host (Finke and Conzelmann, 2005; Finke and Conzelmann, 2003).

Last but not least, host specific innate and adaptive immune response mechanisms can prevent efficient virus replication and limit virus spread. In particular, the type I interferon system has been illustrated to represent a critical determinant of viral host range (for review see Randall and Goodbourn, 2008). Adaptation to efficiently counteract the particular IFN system of a host species is a prerequisite for establishment of an infection. Successful adaptation of bat lyssaviruses to terrestrial animals or humans (as exemplified by RABV) is therefore predicted to involve adaptation to the host's IFN system. The phosphoprotein (P) of RABV has been recently identified as the major player in paralyzing the IFN system in mice and men. Notably, multiple independent mechanisms of P targeting different pathways have been dissected and contribute individually to weaken the IFN system (Rieder and Conzelmann, 2011). In the following, the role of IFN in RABV infection and the critical roles of RABV P are reviewed.

The lyssavirus life style

In contrast to all other rhabdoviruses, which are transmitted by insect vectors, the lyssaviruses are transmitted by direct contact of their mammalian hosts. Entry into

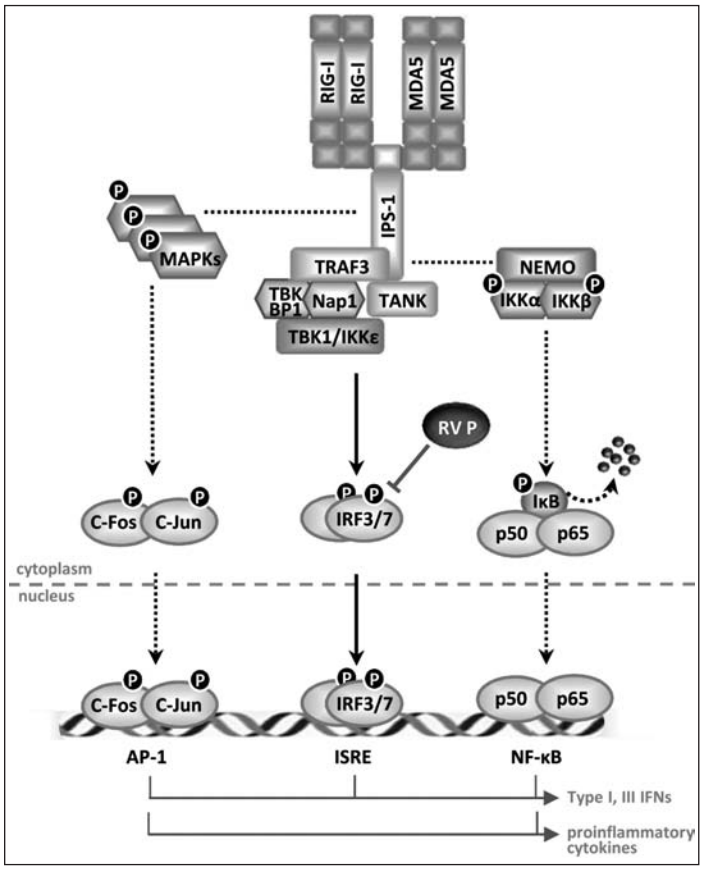

FIGURE 2: *Activation of RIG-I like helicases RIG-I and/or MDA5 by viral RNAs induces IPS-1-dependent activation of IRF3, NF-κB and AP-1, which drive transcription of type I IFNs and proinflammatory cytokines. RABV P can inhibit IFN induction by preventing IRF3 phosphorylation.*

cells involves receptor-mediated endocytosis, membrane fusion at acidic pH, and release of the virus RNP into the cytoplasm of cells, where viral transcription and replication takes place. In the RNP, the viral genome RNA remains completely shielded by the N protein and is not accessible to small cellular compounds, including RNA binding proteins like RNases or small interfering RNAs (Albertini et al., 2008).

Only for transcription and replication is the tight N-RNA clamping opened transiently for access of the polymerase complex (L/P) (Albertini et al., 2008), such that immune stimulatory long dsRNAs are not typically produced (Weber et al., 2006). Transcription of the genome RNA (RNP) starts exclusively at the 3′ end and gives rise to a declining gradient of subgenomic, monocistronic mRNAs with a 5′-cap and 3′-poly(A) tail, and in this respect look like typical cellular mRNAs (for details see Whelan et al., 2004).

Replication of full-length RNPs involves concurrent encapsidation of the nascent RNA into N-RNA and is dependent on high N protein levels. The resulting full-length anti-genome RNP is a template only for production of novel antigenome RNPs, which may serve for secondary transcription, replication, or assembly of novel virions at the plasma membrane. In the absence of N protein, a short 5′-ppp leader RNA is synthesized from the 3′ end of the genome (Leppert et al., 1979), which may represent an emergency shutdown mechanism for replication (Vidal and Kolakofsky, 1989).

RABV and other lyssaviruses are able to enter virtually any cell type in vitro, although primary RABV isolates grow particularly well in neurons, but in vivo infection is characterized by a high neurotropism. The long-range retrograde transport in neurons involves microtubule-mediated passage of membrane vesicles containing complete virus particles (Klingen et al., 2007). After membrane fusion and virus replication in the cell body, new viruses are formed which are transmitted exclusively via synaptic connections to pre-synaptic neurons (Astic et al., 1993) which is being exploited for mapping synaptic connections and neuronal circuits (Ugolini, 2008; Wickersham et al., 2007a, 2007b).

Virulence of lyssaviruses is largely determined by their ability to reach the CNS, which is referred to as "neuroinvasiveness". Avoiding cytotoxicity, innate immunity, and inflammation in order to conserve the integrity of the neuronal network is therefore a key strategy (Finke and Conzelmann, 2005; Dietzschold et al., 2008; Lafon, 2008; Nadin-Davis and Fehlner-Gardiner, 2008; Schnell et al., 2010). Indeed, rabies symptoms appear to arise from neuronal dysfunction rather than from direct or indirect damage (Fu and Jackson, 2005).

The host interferon system

Successful host defense against invading viruses relies on rapid transcriptional activation of type I interferons (IFN-α/β) in virus-infected cells and on IFN-mediated activation of numerous antiviral and immune stimulatory genes in both virus-infected and non-infected cells, to prevent further spread of the virus (Randall and Goodbourn, 2008).

Viruses are recognized as "non-self" (Janeway Jr. and Medzhitov, 2002) by various cellular pattern recognition receptors (PRRs). Prominent viral pathogen associated molecular patterns (PAMPs) are the viral RNAs which can be sensed by various Toll-like receptors like TLR3 (dsRNA) and TLR7/8 (ssRNA), or by RIG-I-like receptors (RLR), including RIG-I, MDA5, and LGP2 (Kawai and Akira, 2010; Yoneyama and Fujita, 2010). Binding of ligands to these receptors results in signalling cascades ultimately leading to transcriptional activation of the single IFN-β gene and the IFN-α gene family comprising a dozen of partially homologous proteins (Calam, 1980). The TLR and RLR pathways for IFN induction merge in the activation of interferon regulatory factors (IRF) 3 and/or IRF7, which are the major transcription factors controlling transcription of type I and III IFN genes (Honda and Taniguchi, 2006; Li et al., 2009).

Virtually all cells can express and secrete IFN-α/β which then acts in an auto- and paracrine fashion by binding to the ubiquitously expressed IFN-α receptor (IFNAR). Expression of the specific IFN type III (IFN-λ) receptors is restricted to certain cell types, including epithelial cells and plasmacytoid dendritic cells (pDC) (Kotenko et al., 2003). Binding triggers the canonical JAK/STAT signal transduction pathways, which activate hundreds of interferon-stimulated genes (ISG). Several of those have antiviral and antiproliferative activities and contribute to the establishment of an antiviral state. In addition, many components

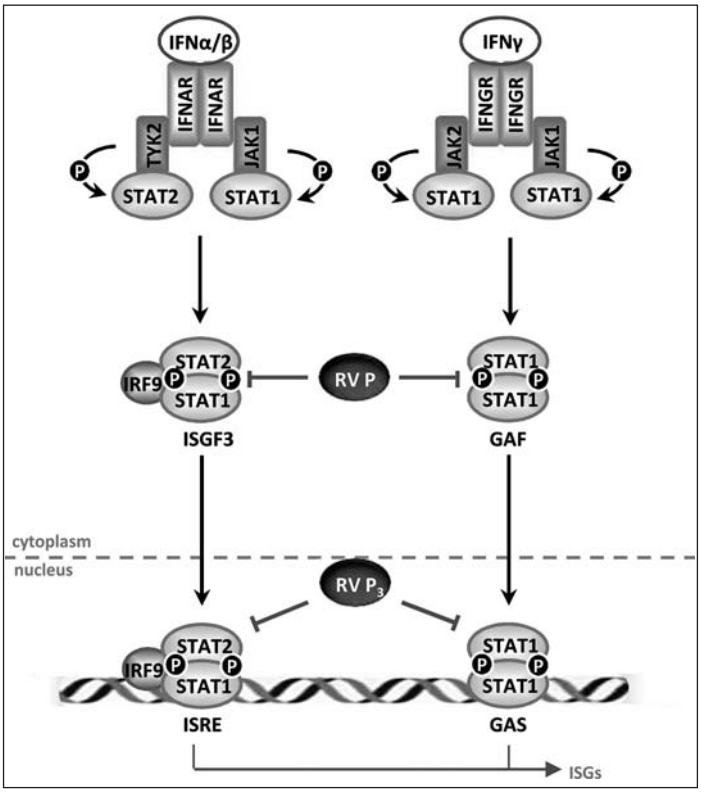

FIGURE 3: *IFN-induced activation of JAK/STAT-signaling. IFN binding to IFN-receptors (IFNAR or IFNGR) leads to tyrosine phosphorylation of STATs by the Janus kinases JAK1, JAK2, or TYK2 as indicated. STAT1/STAT2 heterodimers associate with IRF9 to form the IFN-stimulated gene factor 3 (ISGF3) and STAT1 homodimers represent the γ-activated factor (GAF), which accumulate in the nucleus and drive transcription of ISGs. RABV P inhibits IFN signaling by binding and retention of activated STAT in the cytoplasm, nuclear short P forms may interfere with binding of STATs to DNA.*

of the IFN-inducing pathways are induced, resulting in increased IFN production in virus-infected cells. Moreover, ISGs can stimulate adaptive immunity by stimulating macrophages, increasing major histocompatibility complex class-I (MHC-I) expression, stimulating antibody secretion, and supporting activation of dendritic cells (DC) thereby integrating innate and adaptive immunity (Theofilopoulos et al., 2005).

Host type I IFN is able to limit RABV infection

It is long known that host IFN has an antiviral role in lyssavirus infections. Early evidence for this was obtained by neutralization of host IFN with antibodies, which lead to aggravated disease (Marcovistz et al., 1986), or in experiments in which animals were treated before or simultaneous with RABV infection with IFN, IFN-inducing RNAs like poly (I:C), or IFN-inducing heterologous viruses. In many settings, the exogenous or the induced endogenous IFN could control RABV infection (Marcovistz et al., 1987; Postic and Fenje, 1971; Weinmann et al., 1979). Experiments in transgenic mice deficient for a functional IFN receptor (IFNAR-KO mice) further illustrated that IFN-mediated mechanisms are major factors restricting RABV replication in vivo. These mice were highly susceptible to RABV mutants, which could not cause disease in mice having an intact IFN system (Marschalek et al., 2009; Faul et al., 2008; Chopy et al., 2011).

Nevertheless, natural or experimental infection with RABV and other lyssaviruses regularly leads to induction of detectable IFN and of ISGs in the brain of animals (Stewart and Sulkin, 1966; Sodja, 1975; Marcovistz et al., 1994; Johnson et al., 2006). The observation that attenuated strains induce more IFN than virulent strains (Wang et al., 2005), however, first indicated differential ability of lyssaviruses to counteract the mammalian IFN system and that the IFN antagonistic ability of viruses might correlate with virulence.

Recognition of RABV infection in cells

The RLR RIG-I is emerging as the major PRR for recognition of the RNA of RABV (Hornung et al., 2006) and of other negative strand RNA viruses (Yoneyama et al., 2004; Kato et al., 2006). RIG-I is composed of two N-terminal caspase activation and recruitment domains (CARD) that mediate downstream signalling events, a central ATP-dependent helicase domain, and a short C-terminal domain known as regulatory domain (RD). Purified RNA from virions containing the full-length 5′-ppp RNA, as well as an in vitro transcribed T7 RNA polymerase transcript corresponding to the RABV leader RNA potently activated RIG-I, but not the related MDA5 (Hornung et al., 2006). Further biochemical and structural analyses revealed the C-terminal RIG-I RD domain as the specific binding partner for the 5′-ppp moiety of RABV RNAs (Cui et al., 2008). More recent data indicate that in immune cells MDA5 may also contribute to a certain degree to recognition of various negative strand RNA viruses (Gitlin et al., 2010; Ikegame et al., 2010), including RABV (Faul et al., 2010). Which specific RNAs of negative strand RNA viruses in fact activate RLR in vivo remains a matter of research (for review see Gerlier and Lyles, 2011). RABV with mutations in the N (Masatani et al., 2010), M, and L proteins (unpublished data) were found to induce higher levels of IFN than wt RABV, indicating that properly regulated transcription and encapsidation of viral RNA prevents the generation of RNAs serving as PAMPs.

While VSV RNA appears to be recognized readily by mouse TLR7 (Lund et al., 2004), TLR7-dependent activation of IFN expression by RABV was not observed in isolated human pDC (Hornung et al., 2004). Also in mouse DC, the lack of TLR7 or of TLR3 did not alter IFN expression (Faul et al., 2010), although TLR7 activation was suggested to support a Th1 immune bias and clearance of attenuated RABV (Li et al., 2011). Notably, TLR3 was found to be involved in the generation of RABV Negri bodies (Menager et al., 2009), supporting the previous notion that TLR3 may have proviral rather than antiviral roles (Wang et al., 2004; Le Goffic et al., 2006).

The RABV P protein – a potent IFN antagonist

As lyssaviral RNAs are recognized by PRRs, the viruses must have means to counteract downstream steps of the IFN network. In recent years, the RABV phosphoprotein P emerged as the major player in this task. Intriguingly, RABV P is able to interfere with distinct steps, including IFN induction, IFN signalling, and with the antiviral activity of specific ISGs. RABV P is a 297 aa phosphoprotein containing three structured domains located at the N-terminus, the center of the protein, and the C-terminus, which are separated by two disordered areas providing flexibility (Fig. 1). Like that of other *Mononegavirales*, the RABV P protein is critically involved in RNA synthesis, by acting as a non-catalytic factor of the polymerase complex L-P. RABV P protein also binds to soluble N protein (N^0), thereby allowing specific encapsidation of viral RNA. Moreover, RABV binds various cellular proteins, including dynein light chain (for review see Gerard et al., 2009). In addition to full-length P, truncated P proteins are generated in virus-infected cells from downstream in-frame AUG initiation codons (Chenik et al., 1995). While the longer P forms are located in the cytoplasm, the lack of an N-terminal nuclear export signal in the shorter P forms of the RABV CVS can result in a nuclear localization (Vidy et al., 2007; Moseley et al., 2007).

Inhibition of RLR-mediated IFN induction by the RABV P protein

Activation of RIG-I and MDA5 results in association with the CARD adapter IPS-1, also known as MAVS, VISA, or Cardif (Kawai et al., 2005; Xu et al., 2005; Meylan et al., 2005; Seth et al., 2005), which is located in the outer mitochondrial membrane. This leads to formation of huge MAVS aggregates (Hou et al., 2011) to which numerous signalling molecules associate, including TRAF3, TRADD, TANK, Nap1, and TBKBP1 (for review see Yoneyama and Fujita, 2010). In this complex IRF3 is phosphorylated by the kinases TBK-1 or IKK-ε (Sharma

et al., 2003; Fitzgerald et al., 2003). Phosphorylated IRF3 forms dimers and translocates to the nucleus where it activates the IFN-β promoter (Yoneyama et al., 1998), together with NF-κB and AP1 transcription factors, leading to the production of IFN-β mRNA (Ford and Thanos, 2010) (Fig. 2).

As revealed first by cDNA transfection experiments, the presence of RABV P specifically blocks phosphorylation of IRF3 and IRF7 by the kinases TBK-1 and IKK-ε, such that dimerization and nuclear import, and transcription of IFN-α/β genes is prevented (Brzózka et al., 2005). Attempts to unravel the binding partners of P and the exact mechanisms employed are ongoing.

A corresponding role of P in the virus context was verified by recombinant RABV expressing low amounts of P protein, through P gene shift experiments or IRES-dependent reduction of P protein expression (Brzózka et al., 2005; Marschalek et al., 2009). Whereas in cells infected with the parental RABV (SAD L16) phosphorylation of IRF3 at the critical residue Ser 386 was hardly detectable, the recombinant viruses caused strong activation of IRF3 and IFN induction. The truncated P products may contribute to inhibition of IRF3 activation (Marschalek et al., 2011), though their presence is not crucial, since recombinant RABV expressing only full length P, still effectively counteracted induction of IFN (Brzózka et al., 2005).

Recent mutagenesis experiments allowed identification of a domain in RABV P (*aa 176–186*) required for preventing IRF3 activation, while other functions such as inhibition of STAT signalling (see below) and supporting RNA synthesis remained intact. Importantly, a virus carrying this deletion (SAD ΔInd1) was considerably attenuated after intracerebral injection into mouse brains, providing first evidence that the ability of RABV P to prevent the IFN induction step contributes to RABV pathogenicity in vivo (Rieder et al., 2011).

Inhibition of STAT signalling by the RABV P protein

The antiviral and other biological effects of IFN-α/β are mediated by binding to the respective heterodimeric receptor IFNAR. Upon binding, the intracellular parts of the receptor are phosphorylated by the Janus kinases (JAK) JAK1 and TYK2. STAT1 and STAT2 dock to the phosphorylated IFNAR chains and are phosphorylated at specific tyrosine residues by the JAKs. IRF9 (p48) is recruited to the STAT dimer forming a heteromeric complex referred to as IFN-stimulated gene factor 3 (ISGF3). In case of IFN-γ signalling, only STAT1 is phosphorylated to form the homodimeric γ-activated factor (GAF). The ISGF3 and GAF complexes translocate to the nucleus and bind interferon stimulated response elements (ISRE) and IFN-γ-activated sequences (GAS), respectively, in the promoters of ISG genes (Platanias, 2005) (Fig. 3).

IFN treatment of cells in which a RABV infection has been established previously, does not have major inhibitory effects, indicating viral functions to interfere with ISG induction. Again, the RABV P protein was identified to be responsible. In yeast-two-hybrid experiments, P was found to interact with STAT1 and to prevent STAT1-mediated reporter ISG expression in mammalian cells (Vidy et al., 2005). Moreover, recombinant RABVs expressing low P levels had lost the ability to prevent IFN-α/β and IFN-γ signalling and were sensitive to exogenous IFN (Brzózka et al., 2006). Activation of STAT1 and STAT2 involves phosphorylation of the residues Y701 and Y689, respectively, leading to dimerization and translocation to the nucleus. As revealed by co-precipitation experiments, RABV P binds almost exclusively to tyrosine-phosphorylated STAT1 and STAT2, preventing accumulation of STATs in the nucleus (Brzózka et al., 2006). In non-activated cells the association of P with STATs was not indicated, which may be of advantage in view of the numerous other functions of P during virus replication.

STAT1 and STAT2 binding requires the intact C-terminal domain of P and deletion of the 10 C-terminal aa residues is sufficient to completely abolish binding (Vidy et al., 2005; Brzózka et al., 2006). In addition to full length P, also N-terminally truncated constructs are able to bind and retain STATs in the cytoplasm, including the natural shorter forms of P. Interestingly, though representing a minor fraction of total P products, the short nuclear forms of P from the CVS strain were found to block an intranuclear step in that they prevent STAT1 binding to the promoter of the ISGs (Vidy et al., 2007).

Although recombinant RABV lacking exclusively the STAT binding function could not be generated so far (unpublished experiments), it appears obvious that inhibition of IFN signalling, is as crucial as inhibition of IFN induction. This is supported by the complete attenuation of recombinant viruses expressing low amounts of P (Marschalek et al., 2009). Moreover, a defect of the P protein in preventing STAT signalling in a highly attenuated vaccine RABV strain (Ni-CE) was suggested to contribute to attenuation (Ito et al., 2010).

Interplay of lyssaviruses and antiviral ISGs

Incomplete inhibition of IFN induction and IFN signalling results in the induction of ISGs with potential antiviral activity. While the 2′-5′ OAS/RNaseL system, which leads to degradation of viral and cellular RNAs, appears not to have negative effects on replication of rhabdoviruses (Silverman, 2007), the dsRNA-activated PKR (Kerr et al., 1974; Lebleu et al., 1976), which limits viral translation by phosphorylating the initiation factor eIF-2, emerged as a key component of IFN-induced resistance to VSV (Stojdl et al., 2000; Balachandran et al., 2000). However, PKR seems not to be involved in restricting RABV infection (Blondel et al., 2010). Also human MxA protein, which is a dynamin-like GTPase effective against a broad variety of positive and negative strand RNA viruses, including VSV (Haller and Kochs, 2011) seems to have no effect against RABV (Leroy et al., 2006).

However, data accumulate indicating that specific isoforms (IV and IVa) of the IFN-induced promyelocytic leukemia (PML) protein, also known as tripartite motif protein 19 (TRIM19), play a role in IFN-induced antiviral activities against RABV. PML is a component of nuclear multi-protein complexes known as PML nuclear bodies. In PML-/- MEFs the RABV strain CVS was able to grow to 10–20 fold increased infectious titers (Blondel et al., 2002). Furthermore, overexpression of the PML isoforms

IV and IVa led to reduction of RABV replication (Blondel et al., 2010). Intriguingly, the CVS P binds to PML and retains it in the cytoplasm, suggesting that RABV P is an antagonist of the antiviral PML function as well (Blondel et al., 2002).

Outlook

Although RABV is effectively recognized in cells of mice and men, it is able to grow in these cells because it has evolved means to limit the host IFN response at different steps of the network. Further experimental work comparing the capacities of P proteins from bat-derived lyssaviruses with respect to the individual functions should reveal how well these proteins can restrict the innate immune response of terrestrial hosts and whether, or to which degree these immune-escape functions contribute to the restricted host range of non-rabies lyssaviruses. Most importantly, interdisciplinary research combining experimental biochemical and bioinformatic tools may allow an assessment of how fast bat lyssaviruses may adapt to novel host species and of the risk they pose to human health.

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References

Albertini AA, Schoehn G, Weissenhorn W, Ruigrok RW (2008): Structural aspects of rabies virus replication. Cell Mol Life Sci 65: 282–294.

Astic L, Saucier D, Coulon P, Lafay F, Flamand A (1993): The CVS strain of rabies virus as transneuronal tracer in the olfactory system of mice. Brain Res 619: 146–156.

Badrane H, Bahloul C, Perrin P, Tordo N (2001): Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. J Virol 75: 3268–3276.

Balachandran S, Roberts PC, Brown LE, Truong H, Pattnaik AK, Archer DR, Barber GN (2000): Essential role for the dsRNAdependent protein kinase PKR in innate immunity to viral infection. Immunity 13: 129–141.

Blondel D, Kheddache S, Lahaye X, Dianoux L, Chelbi-Alix MK (2010): Resistance to rabies virus infection conferred by the PMLIV isoform. J Virol 84: 10719–10726.

Blondel D, Regad T, Poisson N, Pavie B, Harper F, Pandolfi PP, De The H, Chelbi-Alix MK (2002): Rabies virus P and small P products interact directly with PML and reorganize PML nuclear bodies. Oncogene 21: 7957–7970.

Botvinkin AD, Poleschuk EM, Kuzmin IV, Borisova TI, Gazaryan SV, Yager P, Rupprecht CE (2003): Novel lyssaviruses isolated from bats in Russia. Emerg Infect Dis 9: 1623–1625.

Boulger LR, Porterfield JS (1958): Isolation of a virus from Nigerian fruit bats. Trans R Soc Trop Med Hyg 52: 421–424.

Brzózka K, Finke S, Conzelmann KK (2005): Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. J Virol 79: 7673–7681.

Brzózka K, Finke S, Conzelmann KK (2006): Inhibition of interferon signaling by rabies virus phosphoprotein P: activation-dependent binding of STAT1 and STAT2. J Virol 80: 2675–2683.

Calam DH (1980): Nomenclature of interferons. Lancet 2: 259.

Chenik M, Chebli K, Blondel D (1995): Translation initiation at alternate in-frame AUG codons in the rabies virus phosphoprotein mRNA is mediated by a ribosomal leaky scanning mechanism. J Virol 69: 707–712.

Chopy D, Detje CN, Lafage M, Kalinke U, Lafon M (2011): The type I interferon response bridles rabies virus infection and reduces pathogenicity. J Neurovirol 17: 353–367.

Cui S, Eisenacher K, Kirchhofer A, Brzózka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP (2008): The C-terminal regulatory domain is the RNA 5′-triphosphate sensor of RIG-I. Mol. Cell 29: 169–179.

Dietzschold B, Li J, Faber M, Schnell M (2008): Concepts in the pathogenesis of rabies. Future Virol 3: 481–490.

Etessami R, Conzelmann KK, Fadai-Ghotbi B, Natelson B, Tsiang H, Ceccaldi PE (2000): Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an in vitro and in vivo study. J Gen Virol 81: 2147–2153.

Faul EJ, Wanjalla CN, McGettigan JP, Schnell MJ (2008): Interferon-beta expressed by a rabies virus-based HIV-1 vaccine vector serves as a molecular adjuvant and decreases pathogenicity. Virology 382: 226–238.

Faul EJ, Wanjalla CN, Suthar MS, Gale M, Wirblich C, Schnell MJ (2010): Rabies virus infection induces type I interferon production in an IPS-1 dependent manner while dendritic cell activation relies on IFNAR signaling. PLoS Pathog 6: e1001016.

Finke S, Conzelmann KK (2003): Dissociation of rabies virus matrix protein functions in regulation of viral RNA synthesis and virus assembly. J Virol 77: 12074–12082.

Finke S, Conzelmann KK (2005): Replication strategies of rabies virus. Virus Res 111: 120–131.

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T (2003): IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 4: 491–496.

Ford E, Thanos D (2010): The transcriptional code of human IFN-beta gene expression. Biochim Biophys Acta 1799: 328–336.

Fraser GC, Hooper PT, Lunt RA, Gould AR, Gleeson LJ, Hyatt AD, Russell GM, Kattenbelt JA (1996): Encephalitis caused by a Lyssavirus in fruit bats in Australia. Emerg Infect Dis 2: 327–331.

Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, Keller B, Kliemt J, Mettenleiter TC, Muhlbach E, Teifke JP, Wohlsein P, Muller T (2011): Novel Lyssavirus in Natterer's Bat, Germany. Emerg Infect Dis 17: 1519–1522.

Fu ZF (2005): Genetic comparison of the rhabdoviruses from animals and plants. Curr Top Microbiol Immunol 292: 1–24.

Fu ZF, Jackson AC (2005): Neuronal dysfunction and death in rabies virus infection. J Neurovirol 11: 101–106.

Gerard FC, Ribeiro EA Jr., Leyrat C, Ivanov I, Blondel D, Longhi S, Ruigrok RW, Jamin M (2009): Modular organization of rabies virus phosphoprotein. J Mol Biol 388: 978–996.

Gerlier D, Lyles DS (2011): Interplay between innate immunity and negative-strand RNA viruses: towards a rational model. Microbiol Mol Biol Rev 75: 468–490.

Gitlin L, Benoit L, Song C, Cella M, Gilfillan S, Holtzman MJ, Colonna M (2010): Melanoma differentiation-associated gene 5 (MDA5) is involved in the innate immune response to *Paramyxoviridae* infection in vivo. PLoS Pathog 6: e1000734.

Haller O, Kochs G (2011): Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res 31: 79–87.

Honda K, Taniguchi T (2006): IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol 6: 644–658.

Hornung V, Ellegast J, Kim S, Brzózka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G (2006): 5′-Triphosphate RNA is the ligand for RIG-I. Science 314: 994–997.

Hornung V, Schlender J, Guenthner-Biller M, Rothenfusser S, Endres S, Conzelmann KK, Hartmann G (2004): Replication-dependent potent IFN-alpha induction in human plasmacytoid dendritic cells by a single-stranded RNA virus. J Immunol 173: 5935–5943.

Hou F, Sun L, Zheng H, Skaug B, Jiang QX, Chen ZJ (2011): MAVS Forms Functional Prion-like Aggregates to Activate and Propagate Antiviral Innate Immune Response. Cell 146: 448–461.

Ikegame S, Takeda M, Ohno S, Nakatsu Y, Nakanishi Y, Yanagi Y (2010): Both RIG-I and MDA5 RNA helicases contribute to the induction of alpha/beta interferon in measles virus-infected human cells. J Virol 84: 372–379.

Ito N, Moseley GW, Blondel D, Shimizu K, Rowe CL, Ito Y, Masatani T, Nakagawa K, Jans DA, Sugiyama M (2010): The Role of Interferon-Antagonist Activity of Rabies Virus Phosphoprotein in Viral Pathogenicity. J Virol 84: 6699–6710.

Janeway CA Jr, Medzhitov R (2002): Innate immune recognition. Annu Rev Immunol 20: 197–216.

Johnson N, McKimmie CS, Mansfield KL, Wakeley PR, Brookes SM, Fazakerley JK, Fooks AR (2006): Lyssavirus infection activates interferon gene expression in the brain. J Gen Virol 87: 2663–2667.

Johnson N, Vos A, Freuling C, Tordo N, Fooks AR, Muller T (2010): Human rabies due to lyssavirus infection of bat origin. Vet Microbiol 142: 151–159.

Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa, Matsuura Y, Fujita T, Akira S (2006): Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441: 101–105.

Kawai T, Akira S (2010): The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11: 373–384.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S (2005): IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol 6: 981–988.

Kerr IM, Brown RE, Ball LA (1974): Increased sensitivity of cell-free protein synthesis to double-stranded RNA after interferon treatment. Nature 250: 57–59.

Klingen Y, Conzelmann KK, Finke S (2007): Double-Labeled Rabies Virus Live tracking of enveloped virus transport. J Virol 82: 237–245.

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP (2003): IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4: 69–77.

Kuz'min IV, Botvinki AD, Khabilov TK (2002): A lyssavirus was isolated from a whiskered bat in Northern Tajikistan. Plecotus et al. 4, 75–81.

Kuz'min IV, Botvinkin AD, Rybin SN, Baialiev AB (1992): [A lyssavirus with an unusual antigenic structure isolated from a bat in southern Kyrgyzstan]. Vopr Virusol 37: 256–259.

Kuz'min IV, Mayer AE, Niezgoda M, Markotter W, Agwanda B, Breiman RF, Rupprecht CE (2010): Shimoni bat virus, a new representative of the *Lyssavirus* genus. Virus Res 149: 197–210.

Lafon M (2005): Rabies virus receptors. J Neurovirol 11: 82–87.

Lafon M (2008): Immune evasion, a critical strategy for rabies virus. Dev Biol (Basel) 131: 413–419.

Le Goffic R, Balloy V, Lagranderie M, Alexopoulou L, Escriou N, Flavell R, Chignard M, Si-Tahar M (2006): Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog 2: e53.

Lebleu B, Sen GC, Shaila S, Cabrer B, Lengyel P (1976): Interferon, double-stranded RNA, and protein phosphorylation. Proc Natl Acad Sci USA 73: 3107–3111.

Leppert M, Rittenhouse L, Perrault J, Summers DF, Kolakofsky D (1979): Plus and minus strand leader RNAs in negative strand virus-infected cells. Cell 18: 735–747.

Leroy M, Pire G, Baise E, Desmecht D (2006): Expression of the interferon-alpha/beta-inducible bovine Mx1 dynamin interferes with replication of rabies virus. Neurobiol Dis 21: 515–521.

Li J, Faber M, Dietzschold B, Hooper DC (2011): The role of toll-like receptors in the induction of immune responses during rabies virus infection. Adv Virus Res 79:115–26.

Li M, Liu X, Zhou Y, Su SB (2009): Interferon-lambdas: the modulators of antivirus, antitumor, and immune responses. J Leukoc Biol 86: 23–32.

Lumio J, Hillbom M, Roine R, Ketonen L, Haltia M, Valle M, Neuvonen E, Lahdevirta J (1986): Human rabies of bat origin in Europe. Lancet 1: 378.

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, Flavell RA (2004): Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc Natl Acad Sci USA 101: 5598–5603.

Marcovistz R, Galabru J, Tsiang H, Hovanessian AG (1986): Neutralization of interferon produced early during rabies virus infection in mice. J Gen Virol 67 (Pt 2): 387–390.

Marcovistz R, Germano PM, Riviere Y, Tsiang H, Hovanessian AG (1987): The effect of interferon treatment in rabies prophylaxis in immunocompetent, immunosuppressed, and immunodeficient mice. J Interferon Res 7: 17–27.

Marcovistz R, Leal EC, Matos DC, Tsiang H (1994): Interferon production and immune response induction in apathogenic rabies virus-infected mice. Acta Virol 38: 193–197.

Marschalek A, Drechsel L, Conzelmann KK (2011): The importance of being short: The role of rabies virus phosphoprotein isoforms assessed by differential IRES translation initiation. Eur J Cell Biol. doi: 10.1016/j.ejcb.2011.01.009.

Marschalek A, Finke S, Schwemmle M, Mayer D, Heimrich B, Stitz L, Conzelmann KK (2009): Attenuation of rabies virus replication and virulence by picornavirus internal ribosome entry site elements. J Virol 83: 1911–1919.

Masatani T, Ito N, Shimizu K, Ito Y, Nakagawa K, Sawaki Y, Koyama H, Sugiyama M (2010): Rabies virus nucleoprotein functions to evade activation of the RIG-I-mediated antiviral response. J Virol 84: 4002–4012.

Menager P, Roux P, Megret F, Bourgeois JP, Le Sourd AM, Danckaert A, Lafage M, Prehaud C, Lafon M (2009): Toll-like receptor 3 (TLR3) plays a major role in the formation of rabies virus Negri Bodies. PLoS Pathog 5: e1000315.

Meredith CD, Prossouw AP, Koch HP (1971): An unusual case of human rabies thought to be of chiropteran origin. S Afr Med J 45: 767–769.

Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J (2005): Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437: 1167–1172.

Moseley GW, Filmer RP, Dejesus MA, Jans DA (2007): Nucleocytoplasmic Distribution of Rabies Virus P-Protein Is Regulated by Phosphorylation Adjacent to C-Terminal Nuclear Import and Export Signals. Biochemistry 46: 12053–12061.

Nadin-Davis SA, Fehlner-Gardiner C (2008): Lyssaviruses: current trends. Adv Virus Res 71: 207–250.

Platanias LC (2005): Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 5: 375–386.

Postic B, Fenje P (1971): Effect of administered interferon on rabies in rabbits. Appl Microbiol 22: 428–431.

Prehaud C, Wolff N, Terrien E, Lafage M, Megret F, Babault N, Cordier F, Tan GS, Maitrepierre E, Menager P, Chopy D, Hoos S, England P, Delepierre M, Schnell MJ, Buc H, Lafon M (2010): Attenuation of rabies virulence: takeover by the cytoplasmic domain of its envelope protein. Sci Signal 3: ra5.

Randall RE, Goodbourn S (2008): Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89: 1–47.

Rieder M, Brzózka K, Pfaller CK, Cox JH, Stitz L, Conzelmann KK (2011): Genetic dissection of interferon-antagonistic functions of rabies virus phosphoprotein: inhibition of interferon regulatory factor 3 activation is important for pathogenicity. J Virol 85: 842–852.

Rieder M, Conzelmann KK (2011): Interferon in rabies virus infection. Adv Virus Res 79: 91–114.

Schneider LG, Barnard BJH, Schneider HP (1985): 1. African viruses: Application of monoclonal antibodies for epidemiological investigations and oral vaccination studies. In: Kuwert E, Merieux C, Koprowski H, Bogel K (eds.), Rabies in the Tropics, 47–59, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.

Schnell MJ, McGettigan JP, Wirblich C, Papaneri A (2010): The cell biology of rabies virus: using stealth to reach the brain. Nat Rev Microbiol 8: 51–61.

Seth RB, Sun L, Ea CK, Chen ZJ (2005): Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122: 669–682.

Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J (2003): Triggering the interferon antiviral response through an IKK-related pathway. Science 300: 1148–1151.

Shope RE, Murphy FA, Harrison AK, Causey OR, Kemp GE, Simpson DI, Moore DL (1970): Two African viruses serologically and morphologically related to rabies virus. J Virol 6: 690–692.

Silverman RH (2007): Viral Encounters with OAS and RNase L during the IFN Antiviral Response. J Virol 81: 12720–12729.

Sodja I (1975): Interferon production by rabies strains isolated from wild rodents. Acta Virol 19: 84–87.

Stewart WE, Sulkin SE (1966): Interferon production in hamsters experimentally infected with rabies virus. Proc Soc Exp Biol Med 123: 650–654.

Stojdl DF, Abraham N, Knowles S, Marius R, Brasey A, Lichty BD, Brown EG, Sonenberg N, Bell JC (2000): The murine double-stranded RNA-dependent protein kinase PKR is required for resistance to vesicular stomatitis virus. J Virol 74: 9580–9585.

Theodorides J (1986): Histoire de la rage, Paris, 289.

Theofilopoulos AN, Baccala R, Beutler B, Kono DH (2005): Type I interferons (alpha/beta) in immunity and autoimmunity. Ann Rev Immunol 23: 307–335.

Ugolini G (2008): Use of rabies virus as a transneuronal tracer of neuronal connections: implications for the understanding of rabies pathogenesis. Dev Biol (Basel) 131: 493–506.

Vidal S, Kolakofsky D (1989): Modified model for the switch from Sendai virus transcription to replication. J Virol 63: 1951–1958.

Vidy A, Chelbi-Alix M, Blondel D (2005): Rabies virus P protein interacts with STAT1 and inhibits interferon signal transduction pathways. J Virol 79: 14411–14420.

Vidy A, El Bougrini J, Chelbi-Alix MK, Blondel D (2007): The nucleocytoplasmic rabies virus P protein counteracts interferon signaling by inhibiting both nuclear accumulation and DNA binding of STAT1. J Virol 81: 4255–4263.

Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA (2004): Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med 10: 1366–1373.

Wang ZW, Sarmento L, Wang Y, Li XQ, Dhingra V, Tseggai T, Jiang B, Fu ZF (2005): Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. J Virol 79: 12554–12565.

Warrell MJ, Warrell DA (2004): Rabies and other lyssavirus diseases. Lancet 363: 959–969.

Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR (2006): Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J Virol 80: 5059–5064.

Weinmann E, Majer M, Hilfenhaus J (1979): Intramuscular and/or intralumbar postexposure treatment of rabies virus-infected cynomolgus monkeys with human interferon. Infect Immun 24: 24–31.

Whelan SP, Barr JN, Wertz GW (2004): Transcription and replication of nonsegmented negative-strand RNA viruses. Curr Top Microbiol Immunol 283: 61–119.

WHO (2005): WHO Expert Consultation on rabies. World Health Organ Tech Rep Ser 931: 1–88.

Wickersham IR, Finke S, Conzelmann KK, Callaway EM (2007a): Retrograde neuronal tracing with a deletion-mutant rabies virus. Nat Methods 4: 47–49.

Wickersham IR, Lyon DC, Barnard RJ, Mori T, Finke S, Conzelmann KK, Young JA, Callaway EM (2007b): Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron 53: 639–647.

Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB (2005): VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol Cell 19: 727–740.

Yoneyama M, Fujita T (2010): Recognition of viral nucleic acids in innate immunity. Rev Med Virol 20: 4–22.

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T (2004): The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 5: 730–737.

Yoneyama M, Suhara W, Fukuhara Y, Fukuda M, Nishida E, Fujita T (1998): Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. EMBO J 17: 1087–1095.

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